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FEATURE ARTICLE

Immediate Early Baculovirus Vectors for Foreign Gene Expression in Transformed or Infected Insect Cells

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One of the major advances provided by recombinant DNA technology is our ability to express cloned genes in a heterologous host, as this permits the isolation of large amounts of foreign gene products for basic studies or direct practical applications. Bacterial systems typically provide high expression levels, but lack eukaryotic protein processing capabilities, and the foreign gene product is often deposited as an insoluble inclusion body. Mammalian systems obviously provide eukaryotic protein processing pathways, but produce foreign proteins at much lower levels and are much more expensive to cultivate than bacteria. The capabilities of the baculovirus-insect cell expression system (1, 2) fall between these two extremes. Baculovirus vectors usually provide very high levels of foreign gene expression, the host cells provide some eukaryotic protein processing capabilities, and, while insect cells remain more expensive to cultivate than bacteria, they can be grown to high density in suspension and recent improvements have reduced the costs of large-scale insect cell fermentation. These proper-

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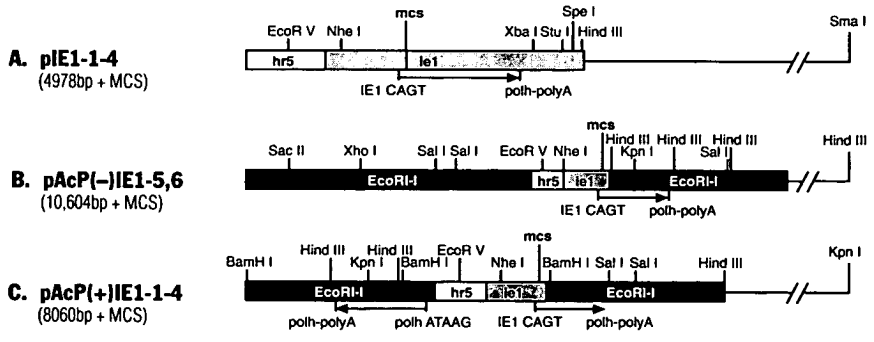

Fig. 1. Immediate early expression plasmids.

The key features of the immediate early expression plasmids described in this study are shown in (A-C). (A) pIE1-1-4 are designed for optimal *ie1*-mediated expression of fused (pIE1-1 and -2) or nonfused (pIE1-3 and -4) proteins in uninfected insect cells. (B) pAcP(–)IE1-5 and -6 are designed for the isolation of occlusion-negative recombinant baculoviruses capable of expressing fused (pAcP(–)IE1-5) or nonfused (pAcP(–)IE1-6) proteins under *ie1* control. (C) pAcP(+)IE1-1-4 are designed for the isolation of occlusion-positive recombinant baculoviruses capable of expressing fused (pAcP(+)IE1-1 and -2) or nonfused (pAcP(+)IE1-3 and -4) proteins under *ie1* control. See Fig. 2 for multiple cloning site (mcs) sequences.

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EXHIBIT 5

The Use of Pellet Paint as a Co-Precipitant in the Ribonuclease Protection Assay

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RNA can be detected and quantified with great sensitivity using the ribonuclease protection assay (RPA). This method, introduced to molecular biologists in 1983, offers several advantages over Northern Blot analysis. First, RPAs are ten to one-hundred times more sensitive. Second, RPAs permit the use of multiple probes of different sizes. By employing internal controls as well as particular probes of concern, scientists can normalize the RNA sample size. However, the protocol generally involves two full days of work. We wanted to find out if we could shorten our protocol by substituting a shorter room temperature precipitation with Pellet Paint, for a longer precipitation at –80°C, and still preserve its sensitivity and reproducibility.

To test Pellet Paint in this procedure, we performed an RPA with two sets of samples, maintaining all conditions constant except the precipitation method. On the first day of the assay, we synthesize and gel-purify ^{32}P-labeled probes before hybridizing overnight with RNA samples. On the second day, we digest unhybridized probe with RNAse, followed by a Proteinase K digestion, a phenol/chloroform extraction, and an ethanol precipitation. At this step, one set of samples was precipitated at room temperature with Pellet Paint for two minutes and spun at room temperature for five minutes. The other set was treated according to our standard protocol; it was precipitated with tRNA at –80°C for fifteen minutes, thawed, and then spun at 4°C for twenty minutes. Both sets were dried and resuspended in loading buffer for examination on a polyacrylamide/urea sequencing gel. In conclusion, Figure 1 shows that protected fragments of a *Xenopus* RNA and two internal controls co-precipitated with Pellet Paint were comparable to those prepared with our standard protocol. In our experience, the use of Pellet Paint streamlines this assay and should prove particularly helpful for processing large numbers of samples.

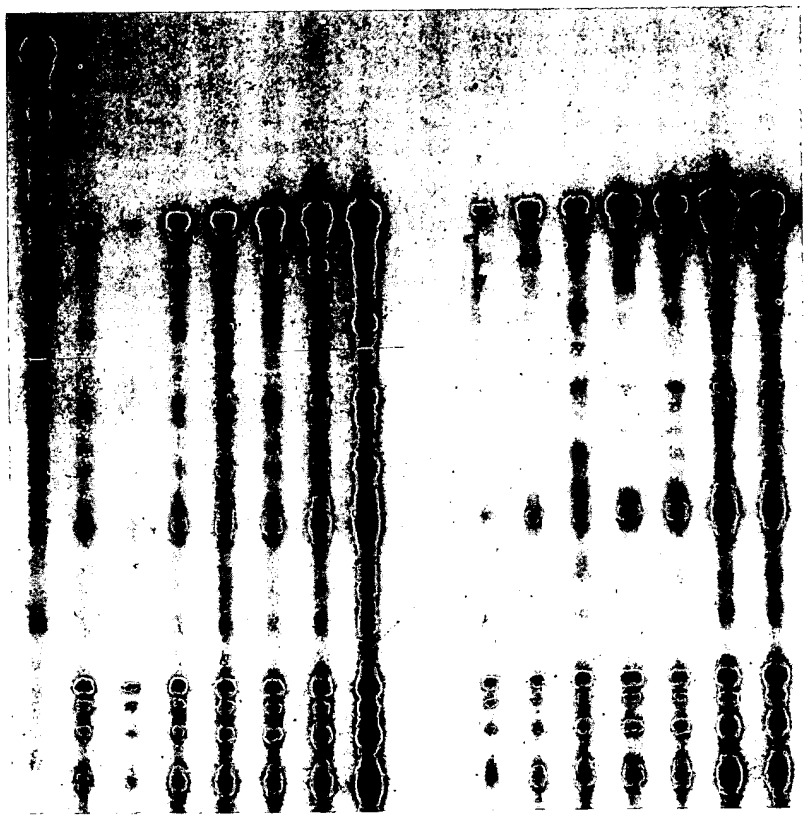

Fig. 1. Autoradiograph of ribonuclease protection assays
Duplicate sets of reactions were carried out under identical conditions. Prior to loading the gel, the set on the right was precipitated with Pellet Paint according to Novagen's protocol. The set on the left was precipitated with tRNA according to a standard protocol.

References

1. For a first report of this method: Zinn, K., DiMaio, D., and Maniatis, T. (1983) *Cell* **34**, 865–879.
2. For a more comprehensive description of this method: Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K., and Green, M.R. (1984) *Nucl. Acids Res.* **12**, 7035–7056.
3. For a description of the protocol used by the authors: Saha, M.S. and Grainger, R.M. (1993) *Molecular Brain Research* **17**, 307–318.

ORDERING INFORMATION

Each 250µl vial of Pellet Paint Co-Precipitant is sufficient for 125 standard precipitations. A vial of qualified 3M Sodium Acetate is also included.

Product	Size	Cat. #	Price
Pellet Paint* Co-Precipitant	250 µl	69049-1	$45

* patent pending